



A new *Neotibicen* cicada subspecies (Hemiptera: Cicadidae) from the southeastern USA forms hybrid zones with a widespread relative despite a divergent male calling song

DAVID C. MARSHALL¹ & KATHY B. R. HILL

Dept. of Ecology and Evolutionary Biology, University of Connecticut, 75 N. Eagleville Rd., Storrs, CT 06269 USA

¹Corresponding author. E-mail: david.marshall@uconn.edu

Abstract

A morphologically cryptic subspecies of *Neotibicen similaris* (Smith and Grossbeck) is described from forests of the Apalachicola region of the southeastern United States. Although the new form exhibits a highly distinctive male calling song, it hybridizes extensively where it meets populations of the nominate subspecies in parapatry, by which it is nearly surrounded. This is the first reported example of hybridization between North American nonperiodical cicadas. Acoustic and morphological characters are added to the original description of the nominate subspecies, and illustrations of complex hybrid song phenotypes are presented. The biogeography of *N. similaris* is discussed in light of historical changes in forest composition on the southeastern Coastal Plain.

Key words: Acoustic behavior, sexual signals, hybridization, hybrid zone, parapatric distribution, speciation

Introduction

The cryptotympanine cicadas of North America have received much recent attention with the publication of comprehensive molecular and cladistic phylogenies and the reassignment of all former North American *Tibicen* Latreille species into new genera (Hill *et al.* 2015; Lee 2015;2016; Sanborn 2015; Sanborn & Heath 2016;2017). The western North American species are now mainly in the genus *Hadoa* Moulds (closely related to *Cacama* Distant and *Cornuplura* Davis), the large-bodied central-eastern USA species are in *Megatibicen* Sanborn and Heath, and the green and black central-eastern species are in *Neotibicen* Hill and Moulds. Occasional new species are continuing to be discovered, in part through analysis of male calling songs (e.g., Cole 2008;2017; Stucky 2013).

In this paper, we document a new *Neotibicen* with a strikingly divergent song and a restricted distribution near the Apalachicola region of Florida, Georgia and Alabama, an area known for high species diversity of some groups (Noss *et al.* 2015). Morphological, acoustic and DNA evidence shows that the new form is closely allied to *Neotibicen similaris* (Smith & Grossbeck) (see note on generic synonymy immediately following the Methods). Furthermore, the two sisters inhabit interlocking ranges and form hybrid zones upon contact, as shown by hybrid song phenotypes. Below, we describe the new form at the subspecies level and include a description of the song of nominate *N. similaris*, since only song characters consistently distinguish the taxa.

Methods

Cicada species were determined in the field by the songs of the males, which are distinctive and facilitate rapid accumulation of distributional data (Marshall *et al.* 1996; Riede 1998). A Garmin GPS V (Olathe, KS), using the WGS84 map datum, was used to estimate locations where males were collected or heard singing (usually within about 200 m of the receiver), or the GPS was later estimated using Google Maps (<http://maps.google.com>). During searching, we drove with the car windows open at ca. 35–45 mph and listened for cicada songs. Even brief

fragments of *Neotibicen similaris* songs are easily detected this way because they contain sound energy above the main frequencies contained in car and wind noise. Records of all cicada species locally present, sometimes with digital audio recordings, were taken every few miles with the car stopped and especially if one taxon was heard after a long period of absence. Cicada specimens were collected as adults with nets during the day or by attracting them to light at night, or by finding fifth-instar nymphs emerging shortly after dark. Specimens collected after ecdysis were pinned with labels identifying the specimen and its separately pinned nymph shell. Before preservation of some specimens, 1–2 legs were removed into 95% ethanol for genetic analysis. These are stored at -20C in the Simon lab collection at the University of Connecticut. Pinned specimens were lodged as noted in the descriptions below.

Cicada songs were recorded in the field using one of several digital recorder/condenser microphone combinations, sometimes together with a Sony (Park Ridge, NJ, USA) PBR330 parabolic reflector. Recorders used included the Sony TCD-D8 (2002 and 2003 only), Marantz (Mahwah, NJ, USA) PMD660, Marantz PMD670, and the Zoom (Ronkonkoma, NY, USA) H4n (in 2012 only), while the microphones used included a Sennheiser (Old Lyme, CT, USA) ME66 short shot gun and a Sennheiser ME62 omnidirectional (both together with the Sennheiser K6 power module). Both Sennheiser microphones have a frequency response from 40–20,000 Hz (+/- 2.5 dB). Songs were sampled at either 44.1 kHz or 48 kHz.

Songs were examined using Raven Pro version 1.4 (Cornell Lab of Ornithology, Ithaca, NY). For analysis, recordings of sufficient quality to measure all characters were selected from throughout the range of both subspecies, and the most clearly resolved song phrase was selected from each track. Because most singing cicadas were mobile and located in tall trees, it was not possible to follow individual cicadas or count the number singing at a given location.

For song descriptions, a *pulse* (*syllable* of Fonseca 2014) is a unit of sound energy that is likely to correspond to a single in- or out-click of a cicada timbal (e.g., Fleming 1975), or possibly synchronized clicks from both timbals (each pulse contains many fundamental sound waves), an *echeme* is a characteristic combination of pulses derived from multiple timbal clicks, and echemes are grouped to form the complete *phrase* of the song. Our field recordings of these species are usually not of sufficient quality to allow unambiguous resolution of features within pulses (e.g., possible separate clicks of timbal ribs).

The main phrase of both subspecies consists of an alternating pattern of low- and high-pitched echemes (see illustrations in Results) that also differ in amplitude, with the nominate subspecies having two parts to the main phrase (here called part I and part II) that differ in the rate of alternation of these components, with part I slower. Parameters measured for both subspecies were as follows: duration of main phrase, separate durations of part I and part II of main phrase (nominate subspecies only), rate of alternation between high- and low-pitched echemes in the main phrase (separately measured for part I and part II of the main phrase in the nominate subspecies), duration of high-pitched echeme, duration of low-pitched echeme, dominant frequency of high-pitched echeme, and dominant frequency of low-pitched echeme (these last four characters measured from the faster-rate part II of the main phrase in the nominate subspecies). Default spectrogram parameters in Raven were used for estimation of dominant frequencies (i.e., Hann window type, window size 5.33 ms, Hop size 2.67 ms, grid spacing 188 Hz). For illustrations, the spectrogram window size was varied from 1.6 to 21.9 ms depending on the temporal resolution required. Recordings were filtered to remove sound energy below approximately 1.8 kHz.

Morphological measurements were made with Vernier calipers or with an ocular micrometer in a Wild M3C stereomicroscope. Statistical tests were conducted in R version 3.2.4 (R Development Core Team 2011). External male genitalia were imaged using an Automontage system (Syncroscopy, Cambridge, UK). For internal male genitalia (primarily the aedeagus), the pygofer was detached after softening and digested in 10% KOH solution overnight at room temperature for clearing. These preparations were washed, stored in 80% ethanol, and imaged with a combination of the stereomicroscope and a LG phone camera held to the left eyepiece. All images were processed using Adobe Photoshop CS5 v12.0.4 (Adobe Systems Incorporated) using the levels, contrast, brightness, sharpen, and auto tone controls as appropriate to improve color replication. Morphological terminology follows Moulds (2005).

Abbreviations for collections referenced in the paper are as follows: Hill and Marshall Collection, currently at the Biodiversity Research Collection at the University of Connecticut (KHDM); Wm T. Davis Collection, Staten Island Museum of Natural Sciences, New York (WTD); American Museum of Natural History, New York (AMNH); National Museum of Natural History, Washington DC (USNM); University of Florida Arthropod

Collection, Gainesville, Florida (FAC); Allen F. Sanborn Collection, Barry University, Florida (AFS); Maxwell S. Moulds Collection, Queensland, Australia (MSM); and the University of Michigan Museum of Zoology (UMMZ).

Results

Synonymy of *Paratibicen* Lee, 2016 and *Neotibicen* Hill and Moulds, 2015. Lee (2016) recently established the genus *Paratibicen* and included only *Cicada similaris* Smith and Grossbeck, which was previously combined with *Neotibicen* Hill and Moulds. The decision was based primarily on genitalic attributes including dorsodistal pygofer shape, two backward-pointing projections of the uncus, the position of attachment of the basal pygofer lobe and the shape of the pygofer in ventral view.

Very soon after the publication of *Paratibicen*, Sanborn and Heath (2017) returned *similaris* to *Neotibicen*, noting that the molecular dataset of Hill *et al.* (2015) does not consistently support *similaris* as the sister lineage to the remainder of *Neotibicen*. In addition, Hill *et al.* (2015) include a cladistic morphological tree showing *similaris* in a derived position within *Neotibicen*, although this is inconclusive because not all of the characters referenced by Lee were included.

In this paper, we also maintain the earlier *Neotibicen* concept and concur that the characters noted by Lee (2016) do not merit the formation of a monotypic genus. Other than the highly distinctive uncus, which was noted by earlier authors (Davis 1922; Hill *et al.* 2015; Smith & Grossbeck 1907), the genitalic characters identified by Lee are subtle and unlikely to be applied consistently (see pygofer images in Supplementary Fig. 1). While the *N. similaris* pygofer in ventral view is somewhat more oblong in shape than in many other *Neotibicen* (which Lee, p. 449, described as "barrel-shaped, with a little widened distal part"), the widest width is near the attachment of the basal lobes in all species. Laterally, the pygofer is similar to that of *N. lyricen* (De Geer) (see also Smith & Grossbeck 1907, Plate III). The dorsodistal margin of the pygofer, which Lee (2016) described as "not lower than distal shoulders", does not always meet this criterion in our specimens (see Supplementary Fig. 1 and Results). Although the "narrowly V-shaped" eighth sternite described by Lee is more acutely angled than in other *Neotibicen*, it is close to that found in *N. davisi harnedi* (Davis) and *N. davisi davisi* (Smith and Grossbeck) (see also Hill *et al.* 2015). Sanborn and Heath (2017) have noted as well that intraspecific variation in the shape of the eighth sternite causes problems for generic definitions based on single specimens. More importantly, however, no features of external morphology consistently separate the remaining *Neotibicen* from *N. similaris*, which resembles *N. lyricen* so closely that the two are commonly confused in collections (Smith and Grossbeck 1907; Davis 1912). *Neotibicen similaris* also shares its habitat (singing from large deciduous and coniferous trees), season of adult appearance (mid to late summer), and singing mode (complex song phrases of less than a minute's duration punctuated by occasional flights to new perches) with many of the *Neotibicen* species. Because *similaris* shares so much of its biology with other members of the genus *Neotibicen*, and differs only in genitalic features that have been long recognized, it is undesirable to separate it from *Neotibicen*, especially in the absence of unambiguous molecular evidence supporting a sister-group relationship.

Neotibicen similaris apalachicola, n. subsp.

Neotibicen nr. *similaris*, Hill *et al.*, 2015: 233, 234, 235, 250.

Neotibicen cf. *similaris*, Hill *et al.*, 2015: 239.

Type locality. Florida, Leon County, rest area on Interstate Highway 10, 0.6 miles east of the Ochlockonee River; latitude 30.485° N, longitude 84.386° W.

Holotype male (Fig. 1): WHITE LABEL: USA: Florida: Leon Co.\\Interstate 10 rest area, W side\\of Tallahassee. 0.6 mi E of the\\Ochlockonee R. 21 July 2008\\30°29.126'N 84° 23.137'W 198ft\\K.Hill & D.Marshall US.FL.TRA. GREEN LABEL: HILL&MARSHALL VOUCHER\\pinned specimen, legs in EtOH\\08.US.FL.TRA.03\\Neotibicen similaris apalachicola\\specimen recorded. Mature specimen attracted to light, recorded singing in cage on 21 July 2008 (see below). Pinned specimen deposited with the AMNH. Right midleg stored with C. Simon EtOH cold-storage tissue collection, University of Connecticut, Storrs, Connecticut, USA.

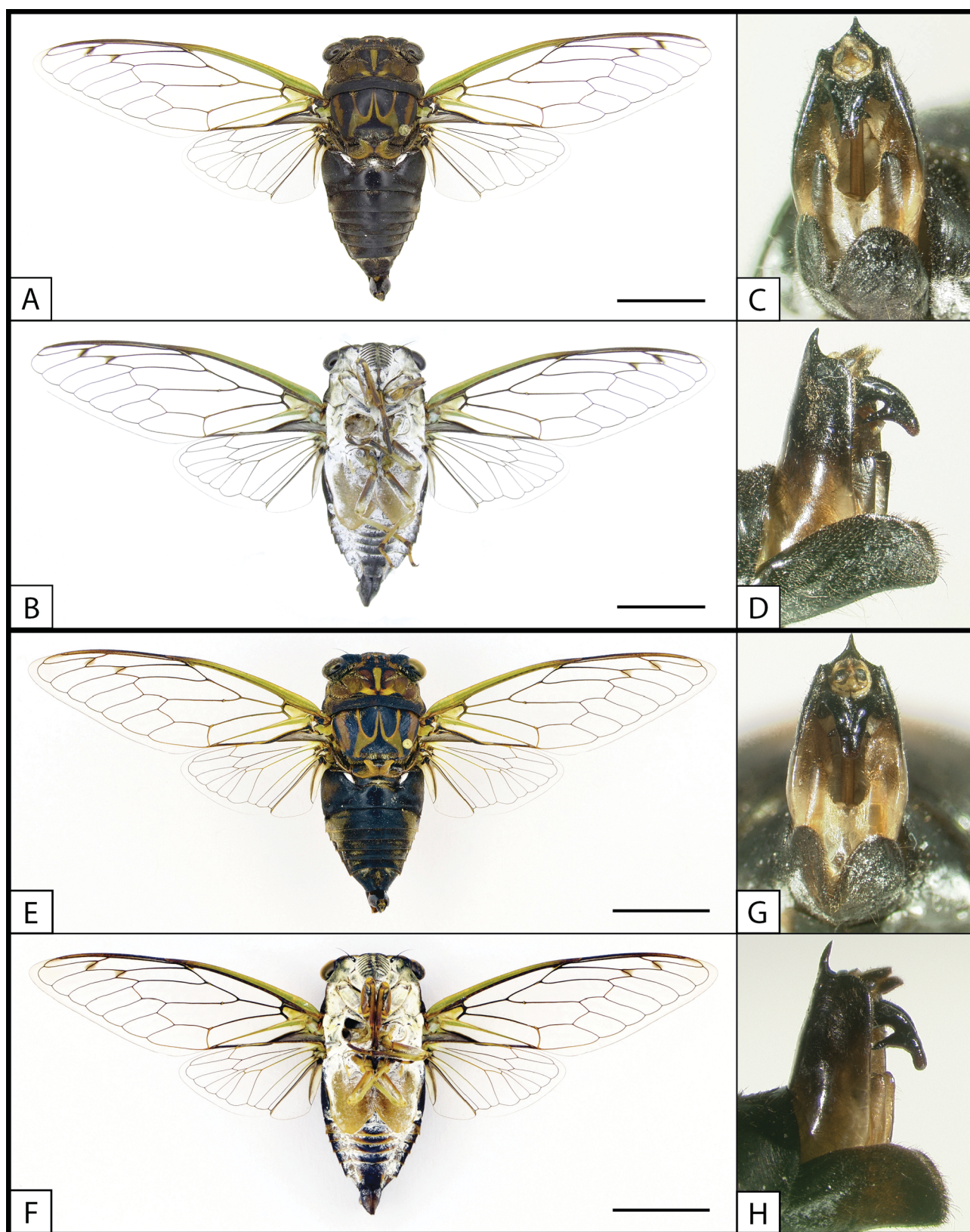


FIGURE 1. A–D, *Neotibicen similaris apalachicola*, **n. subsp.**, dorsal view, ventral view, pygofer ventral view, pygofer lateral view (holotype specimen 08.US.FL.TRA.03). E–H, *N. similaris similaris*, dorsal view, ventral view, pygofer ventral view, pygofer lateral view (specimen 08.US.GA.HAH.01). Scale bars = 12.5 mm.

Paratype specimens: **Florida: Gadsden Co.**—1♂, ~30 mi. W. of Tallahassee, 1 mi. SE of I10 on Rt270A, Flat Creek Rd., 30°36.56N 84°48.59W, 290ft. 02 Jul 2007. D. Marshall & K. Hill. DNA voucher 07.US.FL.FLK.01

(legs in EtOH). Mature male attracted to light. **Jefferson Co.**—1♀, End of Depot St., N. side of Lamont, NW of Aucilla R., 30°22.645'N 83°48.759'W, 80ft. 10 July 2008. K. Hill & D. Marshall. DNA voucher 08.US.FL.LMC.01 (legs in EtOH). Mature specimen. 1♂, Depot St., Lamont, NW of Aucilla River, 30°22.645'N 83°48.759'W, 80ft. 10 July 2008. K. Hill & D. Marshall. DNA voucher 08.US.FL.LMC.02 (legs in EtOH). Genitalic capsule removed and stored separately. **Leon Co.**—1♂, type locality, Interstate 10 rest area, W. side of Tallahassee. 0.6 mi E of the Ochlockonee R., 30°29.126'N 84°23.137'W, 198ft. 10 July 2008. K. Hill & D. Marshall. DNA voucher 08.US.FL.TRA.01 (legs in EtOH). Crushed with abdomen on separate pin. 3♂, same location and collectors, 20 July 2008 (1♂ in WTD, 1♂ in USNM). 1♀, same location and collectors, 20 July 2008, DNA voucher 08.US.FL.TRA.01 (legs in EtOH). 1♀, same location and collectors, 20 July 2008, DNA voucher 08.US.FL.TRA.02 (legs in EtOH). Ecdysis incomplete. 2♀, same location and collectors, 20 July 2008. 1♂, same location and collectors, 21 July 2008, voucher 08.US.FL.TRA.X, genitalic capsule labelled TIB22 removed and stored separately (MSM). 1♀, same location and collectors, 21 July 2008. 2♂, 4♀, same location and collectors, 23 July 2008 (1♂ in AFS, 1♂ 1♀ in FAC, 1♀ in USNM, 1♀ in AMNH). 2♀, same location and collectors, 4 Sep 2008. 2♀, same location, 9 Aug 2010, Rondel Veal. 1♂, 3♀, same location, 13 Aug 2010, K. Hill & D. Marshall. **Wakulla Co.**—1♂, 4.3 mi. NW of US319 on Rt. 267, NW of Hilliardville, 30°18.026'N 84°25.065'W, 83ft. 5 Sep 2008. K. Hill & D. Marshall. US.FL.LEC. Mature males collected singing. All pinned material stored in KHDM collection unless otherwise noted; for collection abbreviations see Methods.

Locations where only recordings or aural records were taken are listed in Supplementary Table 1 along with the specimen records. Note that all type locality specimens except the holotype were collected while emerging and allowed to mature for only 1–2 days before pinning.

Etymology. Named for the Apalachicola River of the Florida panhandle. The subspecies epithet is a noun in apposition and need not agree in gender with its genus following the Code of Zoological Nomenclature (ICZN, 1999), articles 11.9.1.2 and 31.2.1.

Description, holotype male (Fig. 1 A–D). An overall large and dark-colored *Neotibicen*, with black background color and muted brown and green patterning, except where covered in white wax underneath. Small golden or silvery hairs can be seen, where they have not been rubbed off, in various crevices on the dorsal surface and to a lesser extent ventrally.

Head. Mostly black, with small brown patches extending between postclypeus and compound eyes and near ocelli. Compound eyes, viewed dorsally, as wide as or slightly wider than pronotal collar, dark brown or black, with a fringe of short hairs posteriorly. Underside of head covered in white wax except for center of the postclypeus. Postclypeus mostly black with a small brown spot at the top and along the anterior midline, with nine ridges and a central groove. Anteclypeus covered with wax except for brown central midline. Lorum covered with wax. Proboscis light brown at base tending darker towards tip, extending to midway between hind coxae.

Thorax. Pronotum mostly dark brown. Median sulcus muted yellow-green, bordered with black triangle-shaped patches widening anteriorly and with a yellowish brown mark on either side posteriorly, just above the pronotal collar. Pronotal collar black, extreme lateral edges dull green. Mesonotum mostly black, with a thin “crown” pattern varying brown to green, and with the central portion above the cruciform element combining the two inner sigilla to form a large black patch. Lateral color patches, to the outer sides of the lateral sigillas, dark rusty brown. Cruciform elevation light brown, with the center notch black; metanotum brown. Underside of pronotum and mesonotum covered in white wax, this wax at least partially covering the coxae, trochanters and femora.

Legs. With trochanters pale greenish brown, femora mostly brown. Base of tibia pale green on mid and hind legs, pale brown on forelegs, all tips dark brown. Tarsi pale and dark brown with black tarsal claws. Foreleg primary spine angled but not lying flat, secondary spine larger and more erect.

Wings. Slightly longer than body, mainly hyaline. Forewing with basal cell mostly yellow-green, clear near vein CuA, flaps grey, and with only the faintest hint of yellow infuscation otherwise in the membranes. Dark brown infuscations present at the veins joining forewing cells u1/a2 and u2/a3. Basal half of forewing costa green, distal costa brown, ventrally with a black internal border. Vein CuP in forewing green, M and CuA in forewing and CuA and CuP in hindwing greenish brown, otherwise veins mostly black. Hindwing with veins 2A and 3A edged in brownish grey, flaps white.

Abdomen. Tergites glossy black, with bright white wax spots on tergite II on either side above the timbal cover and centrally. Timbal covers black. Underside of abdomen with white wax coating the lateral edges of the

sternites and the opercula, especially the lateral and basal edges. Sternites mostly black, with posterior margins of sternites III–VII medium brown, especially laterally. Sternite VIII forming an upright V-shape in posterior view. Opercula pale brownish green, overlapping for more than half their length. Opercula slightly pointed at tips, reaching the 4th sternite, lateral edges bowed inward slightly where they meet the timbal covers.

Genitalia (Figs. 1B, 1D, 2A). Pygofer black, becoming brown towards base, in ventral view widest at about mid length, with widest width at base less than width of posterior margin of sternite VI; dorsal beak forming a sharp spine; distal shoulders weakly developed and rounded; basal lobes well-developed and visible in lateral view, in ventral view straight with rounded apices, not quite reaching to uncus. Median lobe of uncus black, broadest at base and narrowing evenly to a blunt apex that is almost divided by a deep dorsal suture; laterally with a large pair of black spines either side directed dorsally, their tips just visible dorsally (easily seen laterally). Aedeagus a sclerotized tube with a slightly flared, unsclerotized apex, at rest reaching just beyond the paired uncus spines. Anal styles dark brown.

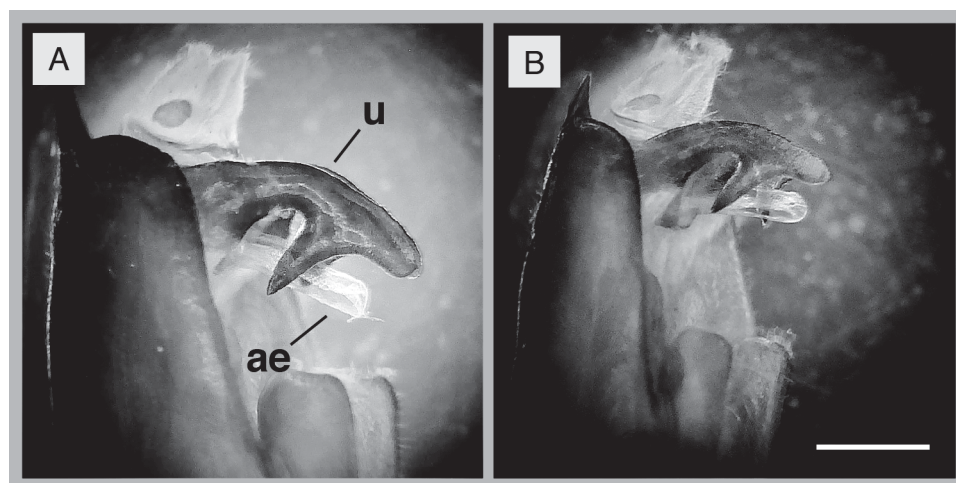


FIGURE 2. Pygofer preparations showing aedeagus (ae) beneath uncus (u): A, *Neotibicen similaris apalachicola*, n. subsp., specimen 08.US.FL.LMC.02; B, *N. similaris similaris*, specimen 08.US.FL.HSC.20. White scale bar = 1 mm.

Song. The following describes a single recorded phrase of the holotype male numbered 08.US.FL.TRA.03 found in voucher recording 08.US.FL.TRA.03.T02.WAV which will be deposited at the online repository BioAcoustica (Baker *et al.*, 2015) and at www.insectsingers.com (Marshall and Hill, 2010). The holotype song phrase consists of approximately 20 s of timbal sound with a frequency range of approximately 2–19 kHz (approximately the limit of the microphone used), containing the following three sections: (1) a leading section consisting of a uniform buzz of increasing intensity approximately 1–2 s long (noted in the field, the holotype recording begins just after this section); (2) a main phrase that alternates sharply and seamlessly between shorter, higher amplitude, high-pitched echemes (0.08–0.13 s each) with sound frequencies mainly from 9–13 kHz and longer, lower amplitude, low-pitched echemes (0.35–0.38 s each) with sound frequencies mainly from 5.5–9.0 kHz (plus a secondary peak near 2.7 kHz)—the single low-pitched echeme could also be described as a series of seamlessly repeated four-pulse echemes; (3) a trailing buzz with sound energy mainly below 9 kHz. The rate of alternation between high- and low-pitched echemes in the main phrase is 2.1 cycles per second. Details of the waveform structures for the latter two parts of the song are as follows: Section (2) above, the main phrase, contains pulses repeated at about 370/sec in the shorter high-pitched echemes and pulses repeated at about 625/sec in the longer low-pitched echemes, the latter visibly grouped into fours based on amplitude patterns. Section (3) contains two-pulse echemes produced at about 240/sec and sometimes alternating in amplitude. In the holotype male phase, a subtle “rattle” lasting about 0.175 s appears near the beginning of the trailing section, suggesting the song pattern of the main phrase of *N. similaris similaris* (see below). The holotype recording is slightly distorted due to the short distance between the specimen and the microphone, which causes an exaggeration and smearing of the frequency spectrum at values over ca. 19 kHz; the important features of the song remain visible. The amplitude decreases at about 2.5 s into the track because the recorder gain was decreased. This recording was made on 21 July 2008 in the rear of an open car following a playback stimulus; ambient temperature was not recorded. Figure 3 shows an example phrase from a higher-quality recording made at a different location.

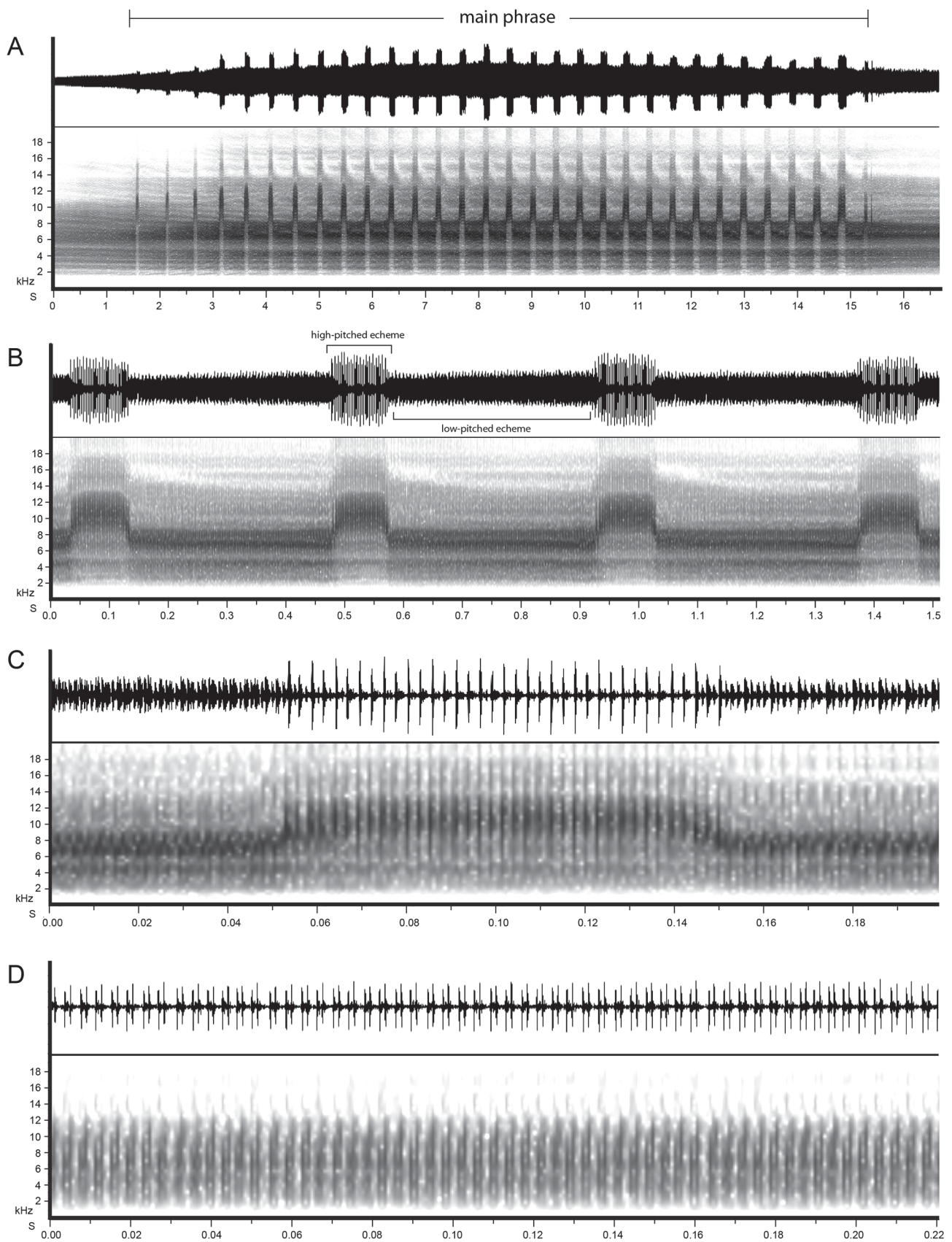


FIGURE 3. *Neotibicen similaris apalachicola*, **n. subsp.**, field recording of male calling song from Wakulla Co., Florida (recording 08.US.FL.BXU.T08.WAV). Each panel shows a waveform above a spectrogram; A, complete song phrase; B, partial zoom of the central portion of the phrase; C, further zoom of one of the four oscillations in B; D, segment of song that precedes the main phrase. Sound energy below 1.8 kHz has been removed.

Morphological variation in male paratypes. Most paratype specimens were not individually recorded singing and some were not collected at the type locality. Subspecies identification in these cases was made on the basis that only *Neotibicen similis apalachicola* songs were heard at and near those locations (see section on Distributions below). Many of the paratypes were preserved only one or two days post-ecdysis and so have only a light covering of wax and fine hairs coating much of the dorsal surface.

Although the male paratypes are overall dark-colored, some have slightly brighter green and brown patterning, and in some the pronotal "crown" is more complete than in others. Many individuals have the veins in the basal half of the forewing and many hind wing veins green or brown (especially on faded specimens). Legs of some specimens are mostly pale green with the tip of each segment tending dark brown. Opercula vary considerably in size and degree of pointedness at the tip, many being almost triangular, some with the tip and lateral portions appearing flattened compared to the inner part which appears to bulge outwards slightly. In all male paratypes the opercula extend at least half-way down the 3rd sternite, and in most they reach at least to the anterior edge of the 4th sternite. The tip of the forewing at the tip of veins R1a and R1b is often lightly infuscated with black, this infuscation sometimes reaching to the apex and slightly around the wing margin. Size measurements are given in Table 1.

Song variation. Measurements of song characters from 13 phrases from throughout the range of *Neotibicen similis apalachicola* are found in Table 2 (see Supplementary Table 1 for source locations). Occasional song phrases from throughout the range, including at the holotype locality, exhibited brief rattles of a small fraction of a second in duration in the transition from the alternating section of the song phrase to the trailing buzz. These features resemble the rapidly alternating section of the song of the nominate subspecies (see below).

Females. Overall similar to males, but tending paler in color with more brown and green, especially ventrally, and with the basal half of the forewing veins usually green or brown (Supplementary Fig. 2). Ovipositor brown to dark brown, not extending beyond sheath. Abdominal sternites variable in color, commonly brown or greenish brown rather than black, sometimes with black centers, and with epipleurites brown to greenish brown. Ninth tergites varying from black adjacent to ovipositor to brown or tan laterally. Size measurements in mm for a sample of 13 female specimens are given in Table 1.

ZooBank registry. urn:lsid:zoobank.org:act:7E149853-51EB-4D61-A96F-B95E34E985C2

TABLE 1. Morphological size measurements (all in mm) for *Neotibicen similis* specimens. Significance levels from two-sided Welch Two Sample t-tests, assuming unequal variance, are given as follows: * = $p < 0.05$, ** = $p < 0.01$.

Character	♂ Mean	Min.	Max.	♀ Mean	Min.	Max.
<i>Neotibicen similis apalachicola</i> , n. subsp. (13♂, 13♀)						
Body length	35.1	32.2	37.7	30.9	29.2	33.8
Forewing length from tip to wing articulation	41.7*	38.1	43.4	41.0	38.5	43.3
Forewing width at widest point	13.3	12.6	14.0	12.8	12.2	14.0
Head width across eyes	14.4*	13.7	15.0	14.0*	13.6	14.9
Thorax width across pronotal collar including flanges	14.2**	12.9	15.1	14.1	13.6	14.9
Opercula length	11.6**	10.9	12.7	N/A	N/A	N/A
Pronotal collar width at either lateral crown point	2.1**	1.8	2.4	2.0	1.8	2.3
<i>Neotibicen similis similis</i> (7♂, 4♀)						
Body length	33.4	29.8	35.4	30.5	29.7	31.0
Forewing length from tip to wing articulation	39.2*	35.6	42.5	39.9	38.0	41.5
Forewing width at widest point	12.8	11.7	13.8	12.9	12.1	13.3
Head width across eyes	13.6*	12.4	14.6	13.5*	13.2	13.8
Thorax width across pronotal collar including flanges	13.2**	12.3	14.3	13.5	12.8	14.2
Opercula length	10.2**	8.7	11.2	N/A	N/A	N/A
Pronotal collar width at either lateral crown point	1.9**	1.6	2.0	1.9	1.8	2.0

TABLE 2. Male calling song measurements of *Neotibicen similaris* specimens. Significance levels from two-sided Welch Two Sample t-tests, assuming unequal variance, are given as follows where characters are comparable: * = $p < 0.05$, ** = $p < 0.01$. The alternation rate character in *N. similaris apalachicola*, **n. subsp.**, was compared to the alternation rate for Part II of the *N. similaris similaris* song.

Character	Mean	Min.	Max.
<i>Neotibicen similaris apalachicola</i> , n. subsp. (13♂)			
Main phrase length (s)	14.4**	9.5	19.3
Rate of alternation between high and low echemes (Hz)	2.3**	2.0	2.9
High pitched echeme duration (s)	0.12**	0.10	0.14
High pitched echeme dominant pitch (kHz)	9.9**	8.8	11.3
Low pitched echeme duration (s)	0.32**	0.24	0.39
Low pitched echeme dominant pitch (kHz)	6.8*	3.8	8.6
<i>Neotibicen similaris similaris</i> (15♂)			
Main phrase length (s)	10.7**	6.3	17.3
Length of part I of main phrase (s)	3.2	1.2	5.8
Rate of alternation between high and low echemes, part I (Hz)	21.7	14.9	24.8
Length of part II of main phrase (s)	7.4	4.6	11.9
Rate of alternation between high and low echemes, part II (Hz)	34.5**	21.9	41.1
High pitched echeme duration (s)	0.01**	0.008	0.015
High pitched echeme dominant pitch (kHz)	8.6**	7.9	9.6
Low pitched echeme duration (s)	0.02**	0.007	0.1
Low pitched echeme dominant pitch (kHz)	5.2*	2.4	7.9

***Neotibicen similaris similaris* (Smith and Grossbeck, 1907)**

Cicada similaris Smith and Grossbeck, 1907: 125.

Rihana similaris Davis, 1912: 262.

Tibicen similaris Van Duzee, 1916: 2, 8, 31–33.

Neotibicen similaris, Hill *et al.*, 2015: 220, 226–227, 249–251.

Paratibicen similaris Lee, 2016: 449, 451, 453.

Type locality. Florida, Nassau County, Fernandina (now called Fernandina Beach). Type specimen deposited in the USNM (see Sanborn 1999).

Specimens examined. **Florida: Alachua Co.**—1♂, High Springs Campgrd., near High Springs at I75 nr jct with Rt. 236, 29°52.458'N 82°32.839'W 38ft. 24 July 2008. K. Hill & D. Marshall US.FL.HSC. Genitalia preparation TIB 26 (MSM). 1♂, same location and collectors, 1 Sep 2008, DNA voucher 08.US.FL.HSC.#20, genitalia dissected. 1♂, same location and collectors, 1 Sep 2008. 2♀, same location and collectors, 02 Sep 2008, one with voucher number 02.US.FL.HSP.01. Marion Co. – 1♀, Rainbow Garden Apartments, Dunnellon, 29°3.070'N, 82°27.428'W, 6m. 14 Sep 2009. K. Hill, D. Marshall, R. Veal. 1♀, same location, R. Veal, 12 Aug 2010. Collected emerging. **Okaloosa Co.**—2♀, 1mi E of Rt189 on Karick Lake Lower Rd., Blackwater River State Forest, 30°53.453'N 86°39.474'W, 84m. 15 Sep 2009. K. Hill & D. Marshall. DNA vouchers 09.US.FL.KLR.01 and 09.US.FL.KLR.02 (legs in EtOH). **St. Johns Co.**—1♂, along Hwy A1A, Matanzas River outlet/Bridge. 5 Aug 1984, F. Huber coll., specimen at UMMZ labelled UMMZ.3. **Taylor Co.**—1♂, 2.6mi N. of US98 on CR14 (Aucilla River Rd), SSW of Lamont, 30°10.422'N 83°53.300'W 37ft. 21 July 2008, K. Hill & D. Marshall. DNA voucher 08.US.FL.AUC.01 (legs in EtOH). **Georgia: Lowndes Co.**—1♂, W. side of Hahira, JCT I75 and Rt122, 30.992°N 83.387°W, 210ft. 24 Jul 2008. K. Hill & D. Marshall. DNA voucher 08.US.GA.HAH.01 (legs in EtOH). **Seminole Co.**—1♂, Cummings Landing Park, near entrance. 2.3 mi S. of Rt. 263, off Rt. 39, 30°47.153'N 84°52.395'W 61ft. 22 July 2008. K. Hill & D. Marshall. US.GA.CUM. Thorax

and abdomen only, no genitalia. **Toombs Co.**—1♂, 0.45mi S of Jarhan Collins Rd on Rt86. 1.2mi S of Rt152. ~10mi ENE of Vidalia, 32.258°N 82.246°W, 276 ft. 25 Jul 2006. K. Hill & D. Marshall. DNA voucher 10.US.GA.SIM.01 (legs in EtOH). For recording-only locality information see Supplementary Table 1. The type specimen of *Neotibicen similaris* was also examined at the USNM, and a dorsal photo is published in Sanborn and Heath (2012). All pinned material is stored in the KHDM collection unless otherwise noted.

Morphological description (see also Fig. 1E–H, 2B). Smith and Grossbeck (1907) described the morphology of the nominate subspecies of *Neotibicen similaris*. Focusing on the unusual uncus, they also noted a male body size of 35 mm and the fact that the "lateral border" of the pronotal collar is largely green, a point made to contrast with *N. lyricen*. These features are shared with *N. similaris apalachicola*. We extend the morphological description to include the characters stated above for *N. similaris apalachicola* with the following modifications noted in the Distinguishing Characters section. Size measurements (in mm) for a sample of seven male specimens of *Neotibicen similaris similaris* are given in Table 1. Note that, as in the preceding section, subspecies identification of these specimens was inferred from geography, with all specimens collected from the region where only *N. similaris similaris* songs were heard.

Song. The following describes a single phrase of a free-flying male of *Neotibicen similaris similaris* recorded in the morning on 29 August 2008 in Nassau County, Florida, at the junction of Highway A1A and Lofton Creek, about 7 air miles west-southwest of Fernandina Beach, the approximate location of the original town of Fernandina (recording 08.US.FL.LCC.T04.WAV) and type locality. This recording will be deposited at the online repository BioAcoustica (Baker et al., 2015) and at www.insectsingers.com (Marshall and Hill, 2010). Much like *N. similaris apalachicola*, the song frequency ranges from around 3 to 19 kHz (approximately the limit of the microphone used) and contains the following three sections: (1) A leading section consisting of a uniform buzz which was audible in the field but is not clearly visible against the background sound in the recording. (2) A main phrase that alternates sharply between high-pitched echemes and low-pitched echemes or sets of echemes; as in *apalachicola*, the high-pitched echemes are also higher in amplitude. The main phrase contains two parts that differ in the rate of alternation and in the temporal pattern. For each cycle in part I (5.7 s in duration), a high-pitched echeme (main energy 8–13 kHz, duration ca. 0.013 s) containing 4–5 pulses is immediately followed by two low-pitched echemes (main energy with peaks at about 4.5 and 6.8 kHz), about 0.016 s and 0.013 s in duration, with the high-pitched echeme seamlessly "slurring" into the first low echeme. The two low-pitched echemes contain shorter pulses that are more numerous and more difficult to resolve than those in the high-pitched echemes. For each cycle in part II of the main phrase, each high-pitched echeme is followed by just one low-pitched echeme. The overall rate of alternation is 14.7 cycles/second in part I and 21.6/sec in part II; note that these values are somewhat slower than in many of our other recordings measured (see below) probably because of a cool morning temperature. Figure 4 shows an example phrase from a higher-quality recording made at a different location.

Song variation. Measurements of song characters from 15 phrases sampled from throughout the range of *Neotibicen similaris similaris* are given in Table 2 (see Supplementary Table 1 for source locations). Note that most song phrases from throughout the range, including at the holotype locality, exhibited isolated irregularly patterned oscillations in the transition from the alternating section of the main phrase to the trailing buzz.

Distinguishing characters. *Neotibicen similaris apalachicola* is easily distinguished from *N. similaris similaris* by features of the male calling song, especially a song phrase containing a single, slow rate of alternation (2–3 cycles/sec) between low-pitched and high-pitched sound, sounding like the word "easy" being slowly repeated. *N. similaris similaris* contains almost the same sound frequencies but alternates very rapidly between low- and high-pitched echemes at more than ten times the rate and increases the rate part-way into the phrase, forming a clacking rattle. The song of *N. similaris apalachicola* is superficially similar to that shared by *N. winnemanna* (Davis) and *N. pruinosus* (Say), which also oscillates between high- and low-pitched sound, but the latter two species produce sound that is mostly below 8 kHz in pitch and the fine-scale structure of the sound is entirely unlike that of *apalachicola* (unpublished data). The song of *N. similaris similaris* superficially resembles the pulsed call phrase of *N. tibicen* (L.), but the rate of oscillation in the latter is only about half that of *similaris* and the fine-scale structure of the song is again entirely different.

Morphologically, *Neotibicen similaris apalachicola* cannot be consistently distinguished from the nominate subspecies, but on average it has a larger body size, a wider pronotal collar, darker forewing infuscation, and longer male opercula (more often extending beyond sternite IV). Although some character means are significantly different, the ranges show broad overlap in all cases (Table 1). Subtle differences exist on average

in the wing venation as well, with the forewing vein r-m between ulnar cell 2 and apical cell 3 more likely to be one-fourth or less of the length of forewing vein m between apical cell 4 and ulnar cell 2. We found no significant differences in the male genitalia of the two subspecies of *N. similaris*.

Both subspecies of *Neotibicen similaris* are easily distinguished from other USA *Neotibicen* species (see Table 3) by the recurved spines protruding from the uncus, which can be viewed without genitalic dissection if the pygofer is gently extruded with a pin while the specimen is soft. In addition, *N. similaris* possesses a partly to entirely black pronotal collar (generally present only in *N. tibicen*, *N. lyricen* and *N. similaris*), a dark pronotum (green in *N. tibicen*), and clear wing membranes (suffused with brown in *N. lyricen*). Aberrant specimens of other eastern *Neotibicen* that usually have green pronotal collars, including *linnei* (Smith and Grossbeck), *winnemanna* (Davis), *robinsonianus* (Davis) and *davisi*, either have bright green markings on the mesothorax (the first three species) or are small, with rounded opercula and a wide head (*N. davisi*). Both subspecies of *N. similaris* are also clearly distinguished from all other USA cicadas by song.

TABLE 3. Distinguishing morphological characteristics of *Neotibicen similaris apalachicola*, **n. subsp.**, and *N. similaris similaris* from other *Neotibicen* species in the southeastern U.S.

	Pronotal collar color	Male genitalia	Mesothorax pattern color	Wing suffused with brown distally?
<i>N. similaris similaris</i> and <i>N. s. apalachicola</i>	black	recurved spine	dull green/brown	no
<i>N. tibicen</i>	black	no spine	green/black	slightly
<i>N. lyricen lyricen</i>	black	no spine	green/brown	slightly (more in <i>I. virescens</i>)
<i>N. linnei</i> , <i>N. winnemanna</i>	green	no spine	green/black	slightly in some
<i>N. robinsonianus</i>	dark green	no spine	green/brown	no
<i>N. davisi</i>	green/brown	no spine	green/brown	no

Ecology and behavior of *Neotibicen similaris* subspecies. Calendar dates for our records of adults and emerging cicadas of *Neotibicen similaris* ranged from 2 July to 28 September. Other sources show *N. similaris* active in Florida from mid-June until late October (Sanborn *et al.* 2008; Walker 2000). Males sing mainly in bright sunshine. The average time of observation of singing cicadas in our study was 12:30 PM, with singing beginning around 8:30 AM and only rarely extending after 6:30 PM. We observed almost no dusk singing, compared to some species like *N. linnei* which are often active around sunset. Males of *N. similaris apalachicola* commonly flew to a new singing station after each song, especially in the morning hours, while males of *N. similaris similaris* appeared more likely to sing several song phrases from one location. When more than one song phrase was sung from a single perch, males of both subspecies produced a continuous low buzz between phrases, as in other *Neotibicen* cicadas. Interestingly, although some cicada species use vertical movements of the abdomen to alter song pitch (e.g., *Magicicada septendecim*, see Allard 1937), no such movement was observed when the holotype male of *N. similaris apalachicola* sang in a cage. Most males heard singing did so from very high stations (almost all over 6m, the maximum reach of our net poles, and most were much higher). Like all *Neotibicen* spp., mature males utter a loud alarm call when disturbed or handled.

Neotibicen similaris cicadas of both subspecies often sang from coniferous trees, which were present at nearly all of the field sites. During collections at the *N. similaris apalachicola* holotype locality from ~9:30 PM to ~12:30 AM on multiple evenings, emerging nymphs were found almost exclusively on large conifers despite the proximity of mature interspersed deciduous trees (Supplementary Fig. 3). Generally, emerging cicadas will climb up the nearest vertical surface, so possibly most of these cicadas had hatched from the branches of the pine trees. However, one female *apalachicola* was collected after she was observed ovipositing in a dead sycamore branch. Furthermore, males of both subspecies also sang from junipers and from deciduous trees like pecans, live oaks, laurel oaks and introduced ginkgo as long as they were large enough. Davis (1918) mentioned male *Neotibicen similaris* singing in small turkey oaks in Florida, and Sanborn and Phillips (2013) have observed *N. similaris* in large deciduous trees (subspecies unknown in both cases).

Distributions of *Neotibicen similaris* subspecies. Song-based records for the *Neotibicen similaris* complex extend across the southeastern USA from Mississippi to North Carolina, including northern and central Florida (Fig. 5). Details of these locations are given in Supplementary Table 1. One record has been published from Louisiana (Sanborn & Phillips 2013), one specimen is known from Pennsylvania (see below), and Davis (1918) included Virginia in the distribution of *N. similaris* without specific information. Sanborn *et al.* (2008, their Fig. 10) showed localities extending the distribution somewhat farther south in Florida, including a disjunct record in Lee Co., FL. The two subspecies inhabit parapatric (interlocking) ranges with hybrid songs evident in areas of contact, as discussed below. The new subspecies *Neotibicen similaris apalachicola* exclusively inhabits a compact section of the Florida panhandle approximately 125 km x 55 km in area, centered on the city of Tallahassee (Fig. 5c). In addition, two corridors of nearly pure *N. similaris apalachicola* populations extend north from Jackson and Leon Counties in Florida and surround a small region of pure *N. similaris similaris* centered on Decatur and Seminole Counties in Georgia. The two corridors of *apalachicola* join again, to the north of these *similaris* populations, and extend farther north to Stewart, Webster, and Sumter Counties in Georgia, after which they expand to the east and west into a region of south-central Alabama and Georgia. The northernmost records of *N. similaris apalachicola* songs are found, to date, in Peach Co. and Johnson Co., GA, and Russell Co., Bullock Co., and Pike Co., AL.

One male in the FAC collection with the label data "PA: BERKS Co./Douglasville//31-VIII-83//F.W.Skillman" was examined for this study. The uncus exhibits the unmistakable inward curving double spines. The only Berks County in the United States is in Pennsylvania. We listened for cicadas around the specified location on the afternoon of 18 September 2009, under cool but sunny conditions, but we heard no *Neotibicen similaris* songs. Additional searching would be worthwhile because this record considerably expands the published range. It is possible that the specimen was mislabeled or collected in Berks County after having been moved there as an egg or a nymph on a transplanted tree (e.g., Chilcote & Stehr 1984).

Hybridization. Putative hybrid songs combining characteristics of *Neotibicen similaris similaris* and *N. similaris apalachicola* were observed in many locations where the subspecies come into contact and apparently interbreed. Examples are shown in Figure 6 and vary from songs more resembling subspecies *similaris* (Fig. 6a), to those more resembling *apalachicola* (Fig. 6b, c), to some with the characters dramatically shifting within the song (Fig. 6d). Fig. 6e shows how the detailed structure of the song in Fig. 6d includes elements of both *apalachicola* (the long high-pitched echeme—compare to Fig. 3c) and *similaris* (alternating short echemes of high and low pitch, compare to Fig. 4d). Note that, while it was our impression in the field that consecutive songs made by the same hybrid male resembled each other more than those of other males, we were unable to confidently track individual singing cicadas, and the degree of song variability in hybrids remains undocumented.

In the southern sector of the range of subspecies *apalachicola* (Fig. 5c), hybrid songs were heard only in a zone approximately 20 km wide separating the core *apalachicola* area from the surrounding populations of true *similaris*. Farther north, in Alabama and Georgia, pure and hybrid populations of both subspecies are less coherently distributed (Fig. 5b); we consider the significance of this pattern in the Discussion. Because our sampling was done rapidly by car, with many sites only briefly checked, our data do not resolve detailed spatial patterns within the hybrid zone, but the region with hybridization was obviously limited relative to the distributions of the parental subspecies. At 388 out of 438 sites where we found *Neotibicen similaris* cicadas, only one subspecies was heard. At 21 sites, normal songs of one or both parental subspecies were heard together with songs exhibiting hybrid influence. At 24 sites, all songs heard showed signs of hybrid influence, although again most of these were only very briefly sampled. The US.AL.GRN (N. side of Greenfield) and US.AL.WFC (Jct. 33/131 E. of Clio) sites were especially notable for the large number of varied hybrid songs heard (see Supplementary Table 1 for location details). There was no significant evidence of coexistence without hybridization: At five sites we noted both parental subspecies present without hybrid songs, but these were samples of less than one minute's duration with hybrid songs recorded at nearby sites.

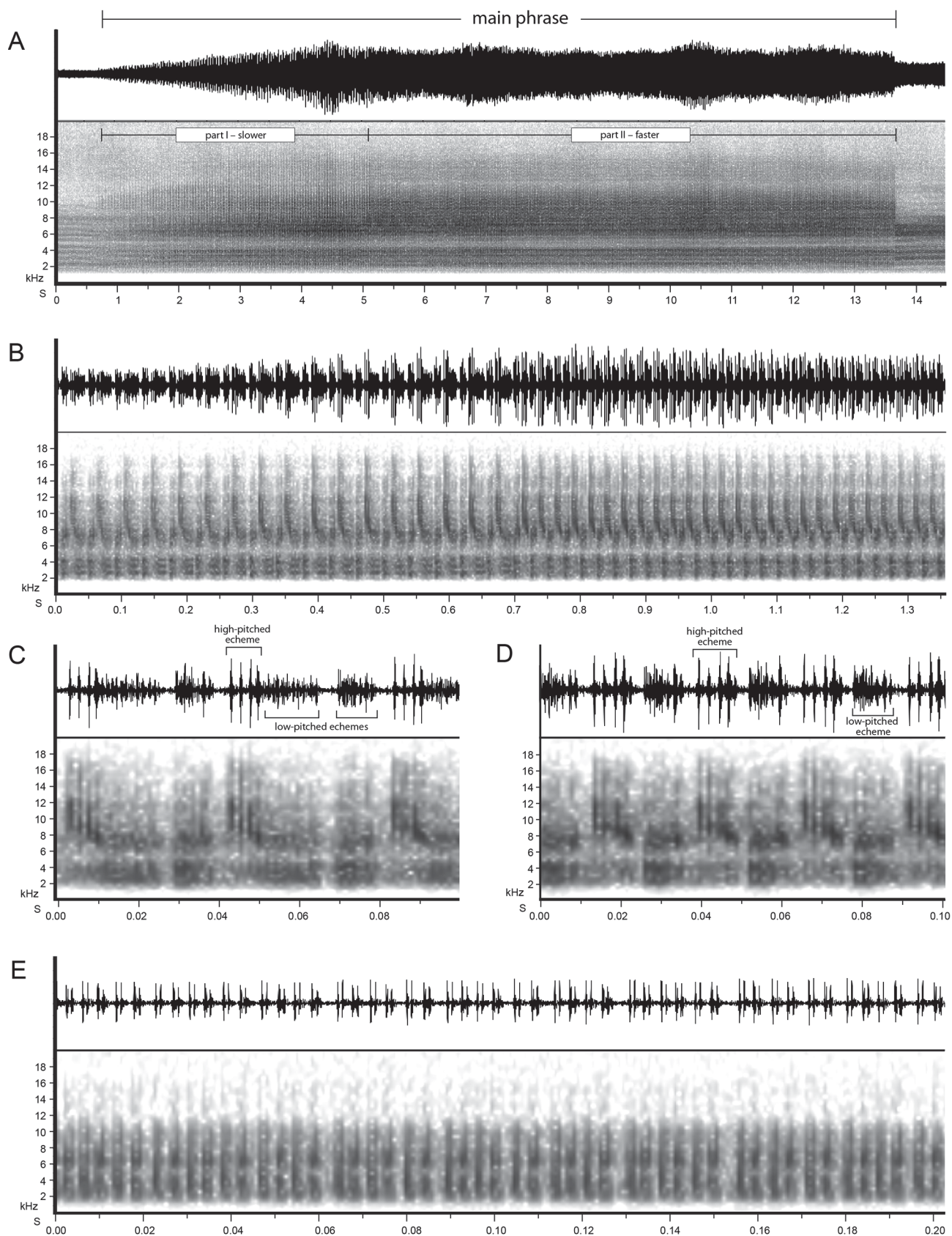


FIGURE 4. *Neotibicen similis similis*, field recording of male calling song from Wakulla Co., Florida (recording 08.US.FL.FOR.T01.WAV). Each panel shows a waveform above a spectrogram; A, complete song phrase, with annotation identifying two parts in the main phrase that differ in rate of alternation between high- and low-pitched echemes; B, zoom centered about 0.48 s into the clip in A, at the transition from part I to part II; C, further zoom of part I; D, further zoom of part II; E, segment of song that precedes and follows the main phrase. Sound energy below 1.8 kHz has been removed.

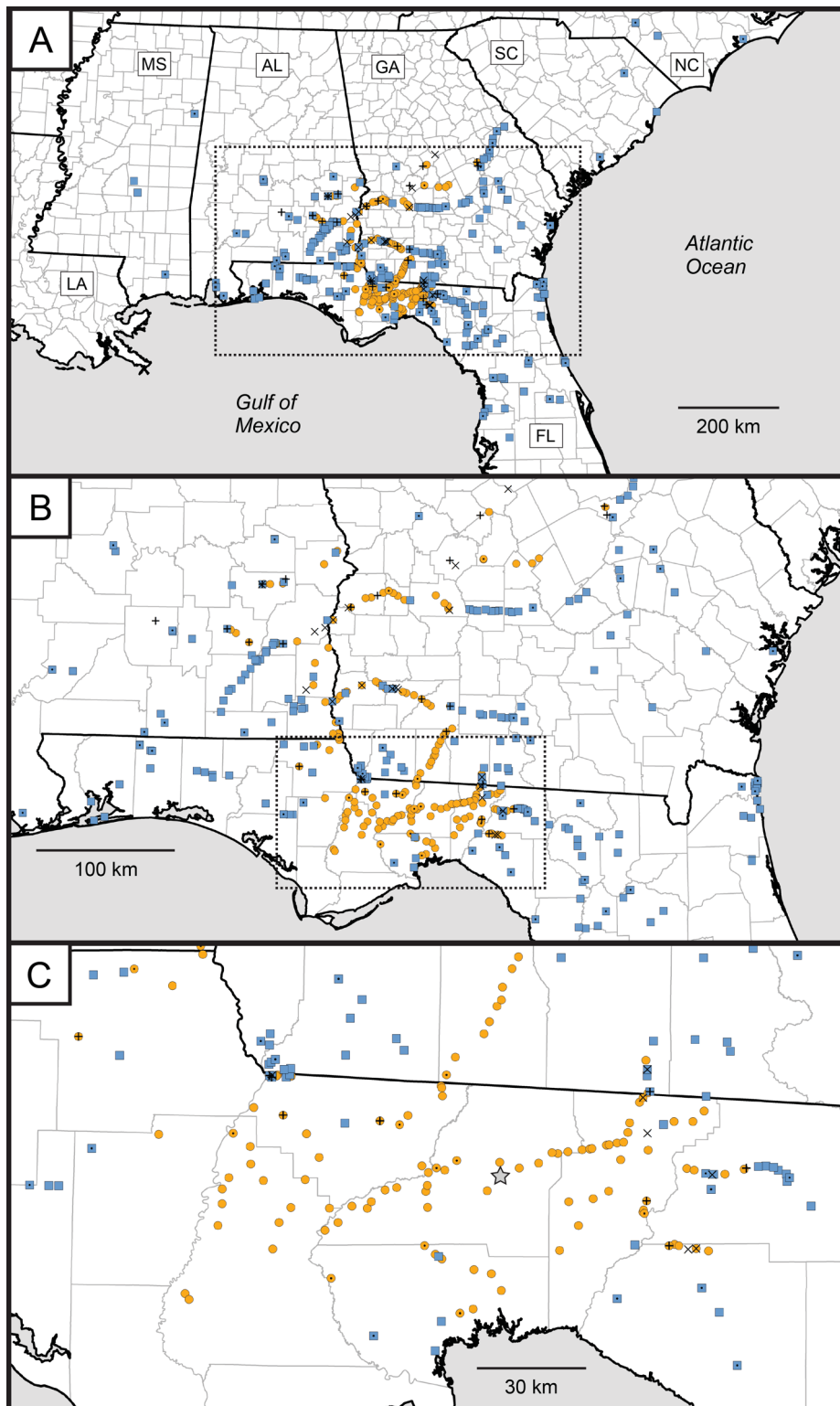


FIGURE 5. Distribution records of *Neotibicen similaris similaris* (blue), *N. similaris apalachicola* n. subsp. (orange), and hybrid cicadas (X and + symbols). Symbols with central dots or a + symbol indicate that a specimen was collected or a voucher recording was made; other records were aurally noted; A, regional scale map of southeastern USA with labeled states outlined in black and with state counties outlined in grey; zoomed section in B is indicated by the dashed line; B, moderate scale map, zoomed section in C is indicated by the dashed line; C, core *N. similaris apalachicola* distribution surrounding Tallahassee, FL (grey star).

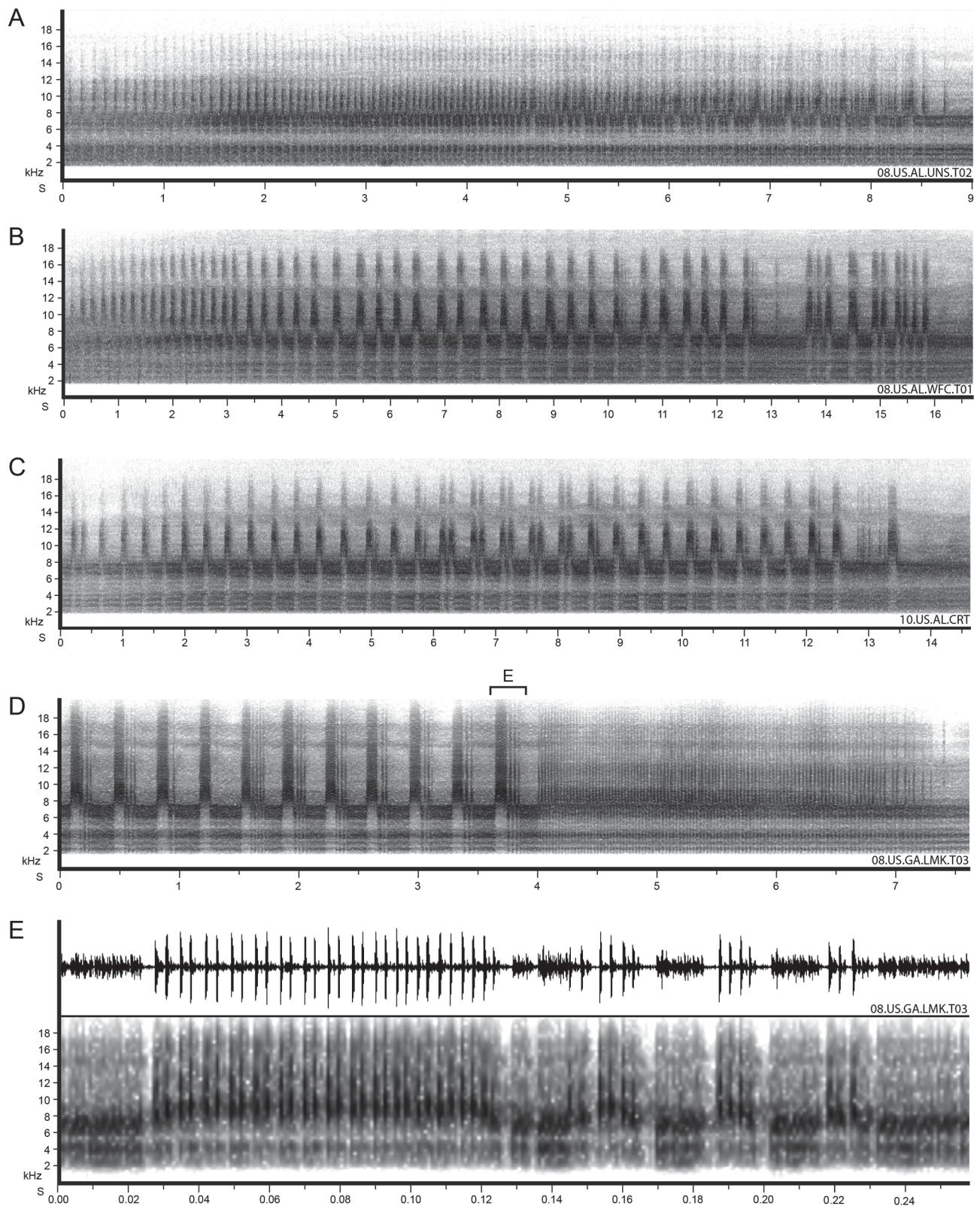


FIGURE 6. Field recordings of putative hybrids between subspecies of *Neotibicen similis*; A–D; spectrograms showing a range of phenotypes exhibiting different combinations of the parental song characters from four different locations (see track ID at bottom right); E, zoomed waveform and spectrogram of the song in D, showing the long high-pitched echeme of *N. similis apalachicola* n. subsp. followed by a set of short echemes matching those of *N. similis similis*. Sound energy below 1.8 kHz has been removed.

Two specimens were collected at sites where hybrid songs were common, one from Stewart Co., GA (DNA voucher 08.US.GA.LMK.01) and one from Mitchell Co., GA (DNA voucher 08.US.GA.FLI.01, see Supplementary Table 1). These specimens are housed at the University of Connecticut. The alarm sound of the first male was recorded.

Discussion

Song divergence and hybridization in *Neotibicen similaris*. The calling songs of acoustically signaling insects such as the cicadas, crickets, and katydids are always distinctive when species live in sympatry and synchrony, while those of related allopatric taxa may or may not differ (Alexander 1962; Otte 1992; Walker 1974). The case of the *Neotibicen similaris* subspecies is striking for the large song difference that exists between such closely related forms that have probably never overlapped in distribution. The songs differ so much to human ears that we failed to recognize the likely close relationship of the two subspecies until we had a specimen in hand. Molecular analysis has confirmed that the taxa are sisters and suggests that their lineages diverged less than 1 Ma (Hill *et al.* 2015), which is consistent with their minimal morphological divergence.

It is remarkable that the *Neotibicen similaris* subspecies perceive each other and readily interbreed despite differences of more than an order of magnitude in some song characters. Sueur and Aubin (2003) have argued, based on inter-male acoustic responses, that pattern differences in broadly similar songs of related cicada species may not be sufficient for distinguishing conspecifics from heterospecifics (Sueur & Aubin 2003; but see Fonseca 2014), but the *Neotibicen similaris* songs are more than subtly divergent, and lesser differences are apparently sufficient for mate discrimination in some sympatric and synchronic cricket species (e.g., Izzo & Gray 2004) and light-flashing fireflies (Lloyd 1966). The songs of the *Neotibicen similaris* subspecies are similar in frequency content, to which cicadas are sensitive (Fonseca *et al.* 2000), and dominant pitch plays a central role in mate recognition in some species (Doolan & Young 1989; Marshall & Cooley 2000; Simmons *et al.* 1971; see also Sueur & Aubin 2002), but this alone is an unsatisfying explanation. Other sympatric and synchronic *Neotibicen* species produce songs that broadly overlap in song pitch (see Supplementary Table 2), with differences not much greater than those observed between the *N. similaris* forms (Table 2). Little has been published on *Neotibicen* mating behavior or mate choice in cicadas, although caged female *Magicicada* appear to choose with threshold-based criteria that exclude only a minority of potential conspecific mates (Cooley & Marshall 2004).

Interbreeding between the song-distinctive *Neotibicen similaris* subspecies helps to demonstrate that even large differences in mate-attracting signals do not necessarily imply a difference in receiver response (Marshall *et al.* 2011; Mendelson & Shaw 2012; Schul 1998), as would be the case if *Neotibicen* cicadas possessed the speciation-facilitating linkages between male song and female preference genes that have been demonstrated for one cricket genus (Wiley *et al.* 2012). Mate discrimination in *Neotibicen* based on limited phenotypic divergence likely requires time for selection to operate, and the contexts that lead to this evolution are not yet generally known. Hybrid zones formed when the potential for gene flow is high, as may be the case here, may rarely lead to reinforcing selection because of the difficulty in maintaining the genetic association between song and preference genes (Rice & Hostert 1993; Servedio & Noor 2003).

Historical biogeography and contact zone formation. Interpreting the complex geographic pattern of song variation observed in *Neotibicen similaris* is difficult because of uncertainty over the habitat preferences of the subspecies and the complicated historical changes in forest composition on the southeastern Coastal Plain. Although spruce trees were present on the southern Coastal Plain with deciduous forest species at the Last Glacial Maximum (LGM, ca. 20 ka) (Delcourt & Delcourt 1977; LaMoreaux *et al.* 2009; Watts *et al.* 1992), boreal forests that would have displaced *Neotibicen similaris* populations apparently did not extend far south of the Appalachians (Webb & Bartlein 1992; Williams *et al.* 2004). Most of the region today inhabited by *Neotibicen similaris* has fluctuated between temperate deciduous- and conifer-dominated forests of varying composition, with the modern southern conifer forests becoming established by the mid-Holocene, around 8.5–4.5 ka depending on location (Delcourt 1977; Schwartz 1994; Watts 1971;1980; Watts *et al.* 1992). If one or both of the *Neotibicen similaris* subspecies are closely linked to southern pines, then their populations may have survived in allopatry during the LGM when coniferous forests were reduced and regions of more open habitat were found interspersed with forest across the southeast (Russell *et al.* 2009). Expansion of southern conifers in the mid Holocene may have led to

population contact and formation of the hybrid zones. This scenario most easily fits the pattern in Florida, where hybrid songs are found only in a comparatively narrow zone of less than 20 km in width surrounding a region of pure *N. similaris apalachicola* (Fig. 5c).

North of the Florida panhandle, hybrid songs appear across a wider region in southern Alabama and Georgia where song phenotypes fluctuate between clusters of pure *Neotibicen similaris similaris* and pure *N. similaris apalachicola* (Fig. 5b). We suspect that this pattern has also formed during range expansions since the LGM: As conditions suitable to *N. similaris* spread back northward, new hybrid populations from northern Florida as well as pure-subspecies populations may have contributed to the recolonization of southern Alabama and Georgia. Thus, corridors with different degrees of hybrid influence could have been established, populations that might have been further modified in their distributions by the forest clearing, regrowth, and composition shifts that have occurred in the modern era (e.g., Frost 2006; Schwartz 1994).

The narrowness of the contact zones in the Florida panhandle is remarkable given that these are large mobile cicadas, although cicadas do have life history features that contribute to low dispersal, including underground juvenile phases of multiple years' duration and brief adult lives of perhaps 1–4 weeks (Boer & Duffels 1996; Campbell *et al.* 2015). Assuming for heuristic purposes a hypothetical life cycle of five years' duration and 1000 generations since mid-Holocene contact, a twenty-kilometer hybrid zone implies only ten meters of net widening of the zone in each direction per generation, which seems implausible. Ecological specialization seems unlikely to maintain this pattern—the subspecies are morphologically similar, variation in elevation and mean precipitation across the region is subtle, and there is no apparent association of the subspecies with different soil types (see Supplementary Fig. 4). However, the distributions of a recently described *Dineutus* beetle species from the Apalachicola region and its closest congener are proposed to follow an ecotone in stream acidity (Gustafson & Miller 2015; Miller & Bergsten 2012), so differentiation in the case of *N. similaris apalachicola* should not be ruled out. The narrowness of the contact zone in Florida may best be explained by a combination of selection against hybrids and limited time since contact was established—perhaps very limited if anthropogenic forest clearing has been a factor.

Additional work is needed to map population patterns within the contact zones, which could shed light on the dynamics of the zone and the nature of selection on hybrids. While the variation in hybrid songs we observed suggests that backcross individuals are present in addition to F1 hybrids (Fig. 6), most of the transition zone appears to consist of pure or nearly pure parental subspecies cicadas together with hybrids, as opposed to a large "hybrid swarm" region composed mainly of backcrossed genotypes. If there is selection against hybrids, the contact zone may behave as a tension zone (Barton & Hewitt 1985), in which case it should tend to migrate to areas of poor habitat quality where population density is lower.

Recent studies have revealed additional complexes of parapatric cicada species and/or subspecies that hybridize in apparent zones of secondary contact (Hertach *et al.* 2016; Marshall *et al.* 2011; Popple 2013). The case presented here is remarkable in that *Neotibicen similaris apalachicola* is almost surrounded by populations of *N. similaris similaris*, so it is possible that the latter contains cryptic lineages from different climatic refugia (one on either side of *N. similaris apalachicola*). This also differs from the common pattern, shown by many southeastern USA animals, of an Atlantic/Florida lineage and a Gulf/Texas lineage (presumably deriving from eastern- and western-refuging Pleistocene populations) meeting approximately where *N. similaris apalachicola* is found (Avise *et al.* 1987; Swenson & Howard 2005; Walker & Avise 1998). Taxa such as *N. similaris apalachicola*, the beetle *Dineutus angusta* (Gustafson & Miller 2015) which is also centered on the Apalachicola region, and the Myola frog (*Litoria myola*) in northeast Queensland, Australia (which also exhibits a small distribution within a "suture zone"), show how differences in ecological and/or historical factors can cause individual taxa to diverge from patterns found in other local community members. Several other southeastern USA cicadas show a different concordant pattern of a "mainland" species or subspecies meeting a Florida peninsula form (e.g., *Cicadetta floridensis*, *Neocicada hieroglyphica johannis*, *Neotibicen lyricen virescens*, and *Neotibicen tibicen australis*) (see maps in Sanborn & Phillips 2013).

The subspecies category in cicada systematics. Many researchers invoke the "separately evolving metapopulation lineage" concept as a basis for recognizing species (De Queiroz 2007) and bring evidence from disparate sources (morphological, genetic, ecological) to bear on their determination. *Neotibicen similaris apalachicola* has evolved a highly distinctive song, probably during one or more past phases of allopatry or parapatry, and on this evidence of diverging evolutionary histories it could be described at the species level under

the De Queiroz concept. The song characters change in a concordant fashion, eliminating one common concern regarding subspecies descriptions (Wilson & Brown 1953). It is likely that we would have named these forms as species if they were not in contact, especially because we would probably have assumed stronger prezygotic isolation. Instead, the boundaries of the two taxa are substantially blurred today, especially in Alabama and Georgia where the transition zone is large (Fig. 5). It is also relevant that we observed minor *similaris*-like "rattles" at the ends of many *apalachicola* songs (including at the holotype location); these could indicate current or past gene flow. Use of the subspecies category here is an acknowledgement that the current situation could be viewed as one structured metapopulation (i.e., one species with subspecies, under the De Queiroz concept) or as two partially but temporarily fusing metapopulations/species that may resume their divergence with loss of the hybrid zone populations during future climate-driven population contractions (Jansson & Dynesius 2002).

Use of the subspecies classification for *Neotibicen similaris apalachicola* is consistent with the approach applied to cicadas in the genera *Pauropsalta* Goding & Froggatt (Popple 2013), *Cicadetta* Kolenati (Hertach *et al.* 2016) and *Thopha* Amyot & Audinet-Serville (Moulds & Hill 2015), although the song differences are greater in our case. In those cases, as here, the described taxa exhibit low levels of genetic and morphological divergence and have geographic relationships ranging from allopatric to parapatric with hybridization. The subspecies concept has been applied in a similar fashion in some terrestrial vertebrate groups of the southeastern US, but not consistently (e.g., Ennen *et al.* 2014; Godwin *et al.* 2014).

Acknowledgements

The authors would like to thank Bill Reynolds (North Carolina State Museum), Allen Sanborn (Barry University), and John Cooley and Chris Simon (University of Connecticut) for help with specimen identification and discussion on North American cicadas. Rondel Veal (Dunnellon, FL) provided assistance in field collection and local natural history observations. Lindsay Popple, Tim McNary, and an anonymous reviewer provided critical comments and suggestions that substantially improved the manuscript. The University of Connecticut Biodiversity Research Collection provided essential curation facilities and access to an Automontage system. Access to external collections was provided by the American Museum of Natural History (Randall Schuh and Ruth Salas), the United States National Museum (Stuart McKamey), and the Florida State Collection of Arthropods. The authors were partially supported by National Science Foundation grants DEB-0955849 and DEB-0720664 to Chris Simon, University of Connecticut.

Literature cited

- Alexander, R.D. (1962) The role of behavioral study in cricket classification. *Systematic Zoology*, 11, 53–72.
<https://doi.org/10.2307/2411453>
- Allard, H.A. (1937) Some observations on the behavior of the periodical cicada *Magicicada septendecim* L. *American Naturalist*, 71, 588–604.
<https://doi.org/10.1086/280746>
- Avice, J.C., Arnold, J., Ball, R.M., Bermingham, E., Lamb, T., Neigel, J.E., Reeb, C.A. & Saunders, N.C. (1987) Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. *Annual Review of Ecology and Systematics*, 18, 489–522.
<https://doi.org/10.1146/annurev.es.18.110187.002421>
- Baker E., Price, B.W., Rycroft, D.D., Hill, J. & Smith, V.S. (2015) BioAcoustica: a free and open repository and analysis platform for bioacoustics. *Database*, 2015, 1–10.
<https://doi.org/10.1093/database/bav054>
- Barton, N.H. & Hewitt, G.M. (1985) Analysis of hybrid zones. *Annual Review of Ecology and Systematics*, 16, 113–148.
<https://doi.org/10.1146/annurev.es.16.110185.000553>
- Boer, A.J. de & Duffels, J.P. (1996) Historical biogeography of the cicadas of Wallacea, New Guinea and the West Pacific: a geotectonic explanation. *Palaeogeography, Palaeoclimatology, Palaeoecology*, 124, 153–177.
[https://doi.org/10.1016/0031-0182\(96\)00007-7](https://doi.org/10.1016/0031-0182(96)00007-7)
- Campbell, M.A., Van Leuven, J.T., Meister, R.C., Carey, K.M., Simon, C. & McCutcheon, J.P. (2015) Genome expansion via lineage splitting and genome reduction in the cicada endosymbiont *Hodgkinia*. *Proceedings of the National Academy of Sciences of the United States of America*, 112, 10192–10199.

<https://doi.org/10.1073/pnas.1421386112>

- Chilcote, C.A. & Stehr, F.W. (1984) A new record for *Magicicada septendecim* in Michigan (Homoptera:Cicadidae). *Great Lakes Entomologist*, 17, 53–54.
- Cole, J.A. (2008) A new cryptic species of cicada resembling *Tibicen dorsatus* revealed by calling song (Hemiptera: Auchenorrhyncha: Cicadidae). *Annals of the Entomological Society of America*, 101, 815–823.
<https://doi.org/10.1093/aesa/101.5.815>
- Cole, J.A. (2017) A new species of *Megatibicen* endemic to Mescalero-Monahans shinnery sands (Hemiptera: Auchenorrhyncha: Cicadidae). *Zootaxa*, 4236 (3), 553–562.
<https://doi.org/10.11646/zootaxa.4236.3.9>
- Cooley, J.R. & Marshall, D.C. (2004) Thresholds or comparisons: mate choice criteria and sexual selection in a periodical cicada, *Magicicada septendecim* (Hemiptera: Cicadidae). *Behaviour*, 141, 647–673.
<https://doi.org/10.1163/1568539042245187>
- Davis, W.T. (1912) A new variety of *Rihana* (*Cicada*) *sayi* Grossbeck (Hemip.). *Entomological News*, 23, 261–262.
- Davis, W.T. (1918) Mississippi cicadas, with a key to the species of the southeastern United States. *Journal of the New York Entomological Society*, 26, 141–155.
- Davis, W.T. (1922) An annotated list of the cicadas of Virginia with description of a new species. *Journal of the New York Entomological Society*, 30, 36–52.
- De Queiroz, K. (2007) Species concepts and species delimitation. *Systematic Biology*, 56, 879–86.
<https://doi.org/10.1080/10635150701701083>
- Delcourt, P.A. (1977) Goshen Springs: Late Quaternary vegetation record for southern Alabama. *Ecology*, 61, 371–386.
<https://doi.org/10.2307/1935195>
- Delcourt, P.A. & Delcourt, H.R. (1977) The Tunica Hills, Louisiana-Mississippi: Late Glacial locality for spruce and deciduous forest species. *Quaternary Research*, 7, 218–237.
[https://doi.org/10.1016/0033-5894\(77\)90038-2](https://doi.org/10.1016/0033-5894(77)90038-2)
- Doolan, J.M. & Young, D. (1989) Relative importance of song parameters during flight phonotaxis and courtship in the bladder cicada *Cystosoma saundersii*. *Journal of Experimental Biology*, 141, 113–131.
- Ennen, J.R., Kalis, M.E., Patterson, A.L., Kreiser, B.R., Lovich, J.E., Godwin, J. & Qualls, C.P. (2014) Clinal variation or validation of a subspecies? A case study of the *Graptemys nigrinoda* complex (Testudines: Emydidae). *Biological Journal of the Linnean Society*, 111, 810–822.
<https://doi.org/10.1111/bij.12234>
- Fleming, C.A. (1975) Acoustic behaviour as a generic character in New Zealand cicadas (Hemiptera: Homoptera). *Journal of the Royal Society of New Zealand*, 5, 47–64.
<https://doi.org/10.1080/03036758.1975.10419379>
- Fonseca, P.J. (2014) Cicada acoustic communication. In: Hedwig, B. (Ed.), *Insect Hearing and Acoustic Communication, Animal Signals and Communication*. 1. Springer-Verlag, Berlin Heidelberg, pp. 101–121.
https://doi.org/10.1007/978-3-642-40462-7_7
- Fonseca, P.J., Munch, D. & Hennig, R.M. (2000) How cicadas interpret acoustic signals. *Nature*, 405, 297–298.
- Frost, C. (2006) History and future of the longleaf pine ecosystem. In: Jose, S., Jokela, E.J. & Miller, D.L. (Eds.), *The Longleaf Pine Ecosystem*. Springer, New York, pp. 9–48.
https://doi.org/10.1007/978-0-387-30687-2_2
- Godwin, J.C., Lovich, J.E., Ennen, J.R., Kreiser, B.R., Folt, B. & Lechowicz, C. (2014) Hybridization of two megacephalic map turtles (Testudines: Emydidae: *Graptemys*) in the Choctawhatchee River drainage of Alabama and Florida. *Copeia*, 2014, 725–742.
<https://doi.org/10.1643/CH-13-132>
- Gustafson, G.T. & Miller, K.B. (2015) The New World whirligig beetles of the genus *Dineutus* Macleay, 1825 (Coleoptera, Gyrinidae, Gyrininae, Dineutini). *ZooKeys*, 2015, 1–135.
<https://doi.org/10.3897/zookeys.476.8630>
- Hertach, T., Puissant, S., Gogala, M., Trilar, T., Hagmann, R., Baur, H., Kunz, G., Wade, E.J., Loader, S.P., Simon, C. & Nagel, P. (2016) Complex within a complex: Integrative taxonomy reveals hidden diversity in *Cicadetta brevippennis* (Hemiptera: Cicadidae) and unexpected relationships with a song divergent relative. *PLoS ONE*, 11, e0165562.
<https://doi.org/10.1371/journal.pone.0165562>
- Hill, K.B.R., Marshall, D.C., Moulds, M.S. & Simon, C. (2015) Molecular phylogenetics, diversification, and systematics of *Tibicen* Latreille 1825 and allied cicadas of the tribe Cryptotympanini, with three new genera and emphasis on species from the USA and Canada (Hemiptera: Auchenorrhyncha: Cicadidae). *Zootaxa*, 3985 (2), 219–251.
<https://doi.org/10.11646/zootaxa.3985.2.3>
- ICZN (1999) *International Code of Zoological Nomenclature*. 4th Edition. The International Trust of Zoological Nomenclature, London, 306 pp.
- Izzo, A.S. & Gray, D.A. (2004) Cricket song in sympatry: species specificity of song without reproductive character displacement in *Gryllus rubens*. *Annals of the Entomological Society of America*, 97 (4), 831–837.
- Jansson, R. & Dynesius, M. (2002) The fate of clades in a world of recurrent climatic change: Milankovitch oscillations and evolution. *Annual Review of Ecology and Systematics*, 33, 741–777.

<https://doi.org/10.1146/annurev.ecolsys.33.010802.150520>

- LaMoreaux, H.K., Brook, G.A. & Knox, J.A. (2009) Late Pleistocene and Holocene environments of the Southeastern United States from the stratigraphy and pollen content of a peat deposit on the Georgia Coastal Plain. *Palaeogeography Palaeoclimatology Palaeoecology*, 280, 300–312.
<https://doi.org/10.1016/j.palaeo.2009.06.017>
- Lee, Y.J. (2015) Description of a new genus, *Auritibicen* gen. nov., of Cryptotympanini (Hemiptera: Cicadidae) with redescrptions of *Auritibicen pekinensis* (Haupt, 1924) comb. nov. and *Auritibicen slocumi* (Chen, 1943) comb. nov. from China and a key to the species of *Auritibicen*. *Zootaxa*, 3980 (2), 241–254.
<https://doi.org/10.11646/zootaxa.3980.2.5>
- Lee, Y.J. (2016) Description of three new genera, *Paratibicen*, *Gigatibicen*, and *Ameritibicen*, of Cryptotympanini (Hemiptera: Cicadidae) and a key to their species. *Journal of Asia-Pacific Biodiversity*, 9, 448–454.
<https://doi.org/10.1016/j.japb.2016.09.002>
- Lloyd, J.E. (1966) Studies on the flash communication system in *Photinus* fireflies. *Miscellaneous Publications of the Museum of Zoology of the University of Michigan*, 130, 1–95.
- Marshall, D.C. & Cooley, J.R. (2000) Reproductive character displacement and speciation in periodical cicadas, with description of a new species, 13-year *Magicicada neotredecim*. *Evolution*, 54, 1313–1325.
<https://doi.org/10.1111/j.0014-3820.2000.tb00564.x>
- Marshall, D.C., Cooley, J.R., Alexander, R.D. & Moore, T.E. (1996) New records of Michigan Cicadidae (Homoptera), with notes on the use of songs to monitor range changes. *Great Lakes Entomologist*, 29, 165–169.
- Marshall, D.C. & Hill, K.B.R. 2010. InsectSingers.com: Song recordings and information on acoustically signaling insects, especially cicadas of the United States and Canada. Available from: <http://www.insectsingers.com> (accessed 2 May 2017)
- Marshall, D.C., Hill, K.B.R., Cooley, J.R. & Simon, C. (2011) Hybridization, mitochondrial DNA taxonomy, and prediction of the early stages of reproductive isolation: Lessons from New Zealand cicadas of the genus *Kikihia*. *Systematic Biology*, 60, 482–502.
<https://doi.org/10.1093/sysbio/syr017>
- Mendelson, T.C. & Shaw, K.L. (2012) The (mis)concept of species recognition. *Trends in Ecology & Evolution*, 27, 421–427.
<https://doi.org/10.1016/j.tree.2012.04.001>
- Miller, K.B. & Bergsten, J. (2012) Phylogeny and classification of whirligig beetles (Coleoptera: Gyrinidae): relaxed-clock model outperforms parsimony and time-free Bayesian analyses. *Systematic Entomology*, 37, 706–746.
<https://doi.org/10.1111/j.1365-3113.2012.00640.x>
- Moulds, M.S. (2005) An appraisal of the higher classification of cicadas (Hemiptera: Cicadoidea) with special reference to the Australian fauna. *Records of the Australian Museum*, 57, 375–446.
<https://doi.org/10.3853/j.0067-1975.57.2005.1447>
- Moulds, M.S. & Hill, K.B.R. (2015) Phylogeny for the tribe Thophini (Cicadoidea: Cicadidae) with the description of a new subspecies of *Thopha sessiliba* Distant from Western Australia. *Records of the Australian Museum*, 67, 55–66.
<https://doi.org/10.3853/j.2201-4349.67.2015.1634>
- Noss, R.F., Platt, W.J., Sorrie, B.A., Weakley, A.S., Means, D.B., Costanza, J. & Peet, R.K. (2015) How global biodiversity hotspots may go unrecognized: lessons from the North American Coastal Plain. *Diversity and Distributions*, 21, 236–244.
<https://doi.org/10.1111/ddi.12278>
- Otte, D. (1992) Evolution of cricket songs. *Journal of Orthoptera Research*, 1, 25–49.
<https://doi.org/10.2307/3503559>
- Popple, L.W. (2013) A revision of the *Pauropsalta annulata* Goding & Froggatt species group (Hemiptera: Cicadidae) based on morphology, calling songs and ecology, with investigations into calling song structure, molecular phylogenetic relationships and a case of hybridisation between two subspecies. *Zootaxa*, 3730 (1), 1–102.
<https://doi.org/10.11646/zootaxa.3730.1.1>
- RDevelopmentCoreTeam. (2011) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available from: <http://www.r-project.org/> (accessed 2 May 2017)
- Rice, W.R. & Hostert, E.E. (1993) Laboratory experiments on speciation: What have we learned in 40 years? *Evolution*, 47, 1637–1653.
<https://doi.org/10.2307/2410209>
- Riede, K. (1998) Acoustic monitoring of Orthoptera and its potential for conservation. *Journal of Insect Conservation*, 2, 217–223.
<https://doi.org/10.1023/A:1009695813606>
- Russell, D.A., Rich, F.J., Schneider, V. & Lynch-Stieglitz, J. (2009) A warm thermal enclave in the Late Pleistocene of the South-eastern United States. *Biological Review*, 84, 173–202.
<https://doi.org/10.1111/j.1469-185X.2008.00069.x>
- Sanborn, A.F. (1999) Cicada (Homoptera: Cicadoidea) type material in the collections of the American Museum of Natural History, California Academy of Sciences, Snow Entomological Museum, Staten Island Institute of Arts and Sciences, and the United States National Museum. *Florida Entomologist*, 82, 34–60.
<https://doi.org/10.2307/3495835>
- Sanborn, A.F. (2015) New combinations for six species belonging to Cryptotympanini Handlirsch (Hemiptera: Cicadidae),

- former members of the genus *Tibicen* Latreille, 1825. *Zootaxa*, 4027 (3), 447–50.
<https://doi.org/10.11646/zootaxa.4027.3.9>
- Sanborn, A.F. & Heath, M.S. (2012) *The cicadas (Hemiptera: Cicadoidea: Cicadidae) of North America north of Mexico*. Entomological Society of America, Lanham, MD, 227 pp.
- Sanborn, A.F. & Heath, M.S. (2016) *Megatibicen* n. gen., a new North American cicada genus (Hemiptera: Cicadidae: Cicadinae: Cryptotympanini). *Zootaxa*, 4168, 577–582.
<https://doi.org/10.11646/zootaxa.4168.3.10>
- Sanborn, A.F. & Heath, M.S. (2017) Priority and synonymy of some North American cicada genera (Hemiptera: Cicadidae: Cicadinae: Cryptotympanini). *Zootaxa*, 4243, 377–382.
<https://doi.org/10.11646/zootaxa.4243.2.8>
- Sanborn, A.F. & Phillips, P. (2013) Biogeography of the cicadas (Hemiptera: Cicadidae) of North America, north of Mexico. *Diversity*, 5, 166–239.
<https://doi.org/10.3390/d5020166>
- Sanborn, A.F., Phillips, P.K. & Gillis, P. (2008) The cicadas of Florida (Hemiptera: Cicadoidea: Cicadidae). *Zootaxa*, 1916, 1–43.
- Schul, J. (1998) Song recognition by temporal cues in a group of closely related bushcricket species (genus *Tettigonia*). *Journal of Comparative Physiology A Sensory Neural and Behavioral Physiology*, 183, 401–410.
<https://doi.org/10.1007/s003590050266>
- Schwartz, M.W. (1994) Natural distribution and abundance of forest species and communities in northern Florida. *Ecology*, 75, 687–705.
<https://doi.org/10.2307/1941727>
- Servedio, M.R. & Noor, M.A.F. (2003) The role of reinforcement in speciation: Theory and data. *Annual Review of Ecology, Evolution, and Systematics*, 34, 339–364.
<https://doi.org/10.1146/annurev.ecolsys.34.011802.132412>
- Simmons, J.A., Weaver, E.G., Strother, W.F., Pylka, J.M. & Long, G.R. (1971) Acoustic behavior of three sympatric species of 17-yr cicadas. *Journal of the Acoustical Society of America*, 49, 93.
<https://doi.org/10.1121/1.1976148>
- Smith, J.B. & Grossbeck, J.A. (1907) Studies in certain cicada species. *Entomological News*, 18, 116–129.
- Stucky, B. (2013) Morphology, bioacoustics, and ecology of *Tibicen neomexicensis* sp. n., a new species of cicada from the Sacramento Mountains in New Mexico, U.S.A. (Hemiptera, Cicadidae, *Tibicen*). *ZooKeys*, 337, 49–71.
<https://doi.org/10.3897/zookeys.337.5950>
- Sueur, J. & Aubin, T. (2002) Acoustic communication in the Palearctic red cicada, *Tibicina haematodes*: chorus organisation, calling-song structure, and signal recognition. *Canadian Journal of Zoology*, 80, 126–136.
<https://doi.org/10.1139/z01-212>
- Sueur, J. & Aubin, T. (2003) Specificity of cicada calling songs in the genus *Tibicina* (Hemiptera: Cicadidae). *Systematic Entomology*, 28, 481–492.
<https://doi.org/10.1046/j.1365-3113.2003.00222.x>
- Swenson, N.G. & Howard, D.J. (2005) Clustering of contact zones, hybrid zones, and phylogeographic breaks in North America. *American Naturalist*, 166, 581–591.
<https://doi.org/10.1086/491688>
- USGS (2016) Mineral Resources On-Line Spatial Data. Geologic Maps of US States. Vol. 2016. Available from: <https://mrdata.usgs.gov/> (accessed 2 May 2017)
- Van Duzee, E.P. (1916) *Check list of Hemiptera (excepting the Aphididae, Aleurodidae and Coccidae) of America, north of Mexico*. New York Entomological Society, New York, 111 pp.
- Walker, D. & Avise, J.C. (1998) Principles of phylogeography as illustrated by freshwater and terrestrial turtles in the southeastern United States. *Annual Review of Ecology and Systematics*, 29, 23–58.
<https://doi.org/10.1146/annurev.ecolsys.29.1.23>
- Walker, T.J. (1974) Character displacement and acoustic insects. *American Zoologist*, 14, 1137–11150.
<https://doi.org/10.1093/icb/14.4.1137>
- Walker, T.J. (2000) Seasonal occurrence of cicadas in Alachua County, Florida. Available from: <http://entnemdept.ifas.ufl.edu/walker/buzz/c700f12.htm>. (accessed 17 December 2016)
- Watts, W.A. (1971) Postglacial and interglacial vegetation history of southern Georgia and central Florida. *Ecology*, 52, 676–690.
<https://doi.org/10.2307/1934159>
- Watts, W.A. (1980) Late-Quaternary vegetation history at White Pond on the inner coastal plain of South Carolina. *Quaternary Research*, 13, 187–199.
[https://doi.org/10.1016/0033-5894\(80\)90028-9](https://doi.org/10.1016/0033-5894(80)90028-9)
- Watts, W.A., Hansen, B.C.S. & Grimm, E.C. (1992) Camel Lake: a 40,000-yr record of vegetational and forest history from northwest Florida. *Ecology*, 73, 1056–1066.
<https://doi.org/10.2307/1940180>
- Webb, T.I. & Bartlein, P.J. (1992) Global changes during the last 3 million years: climatic controls and biotic responses. *Annual*

Review of Ecology and Systematics, 23, 141–173.

<https://doi.org/10.1146/annurev.es.23.110192.001041>

Wiley, C., Ellison, C.K. & Shaw, K.L. (2012) Widespread genetic linkage of mating signals and preferences in the Hawaiian cricket *Laupala*. *Proceedings of the Royal Society Biological Sciences Series B*, 279, 1203–1209.
<https://doi.org/10.1098/rspb.2011.1740>

Williams, J.W., Shuman, B.N., Webb, T., Bartlein, P.J. & Leduc, P.L. (2004) Late-Quaternary Vegetation Dynamics in North America: Scaling from Taxa to Biomes. *Ecological Monographs*, 74, 309–334.
<https://doi.org/10.1890/02-4045>

Wilson, E.O. & Brown, J.W.L. (1953) The subspecies concept and its taxonomic application. *Systematic Zoology*, 2, 97–111.
<https://doi.org/10.2307/2411818>